Standard Operating Procedure for:

ANALYSIS OF ENVIRONMENTAL TRACERS IN WATER SAMPLES USING GC/MS/MS

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1. SCOPE AND APPLICATION

1.1. This method is applicable to the determination of seven environmental tracers in water samples as listed in Table 1. Method Detection Limits and Practical Quantitation Limits yielded by this method are also provided in Table 1.

1.2. This method is based on EPA 8270D (see 15.1) Laboratory Information Management System (LIMS) test: W-TR-TQ.

1.3. This Standard Operation Procedure is restricted to use by an analyst experienced in the use of an Agilent 7000 GC/MS triple quadrupole system, and in the use of appropriate computer programs. The minimum qualifications required:

- B.S. in Chemistry or Biochemistry with minimum one year of professional chemical or biochemical experience
- Knowledge of principles and practice of gas chromatography/mass spectrometry
- Ability to perform basic laboratory mathematics
- Knowledge of the Chemstation and Masshunter GC/MS softwares and DEP LIMS.

2. SUMMARY OF THE METHOD

Water samples are extracted using the appropriate procedures listed in the Preparation SOP- SV-001-3. The extract is analyzed using an Agilent 7000 GC/MS triple quadrupole system operating in the Multiple Reaction Monitoring (MRM) mode. Qualitative identification of the parameters in the extract is performed using the retention time and the relative abundance of the characteristic MRM transitions. Quantitative analysis is performed using an internal standard technique.

3. INTERFERENCES

3.1. Raw GC/MS QQQ data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

3.2. Contamination by carryover can occur whenever high-level and low-level samples are analyzed sequentially. To reduce carryover, rinse the syringe several times with solvent between samples. It may be necessary to analyze one or more solvent blanks between samples and even change out the syringe in extreme cases.

3.3 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must routinely demonstrate to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 4.10. Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by rinsing with DI water. No detergent should be used since they commonly contain Galaxolide/Tonalide as fragrances. The glassware should then be drained dry, and heated in a muffle furnace at 550 °C for 8 hours. Some thermally stable materials may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may supplement the muffle furnace heating. Volumetric glassware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil. The use of high purity reagents and solvents helps to minimize interference

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problems. Purification of solvents by distillation in all-glass systems may be required or irradiation with UV light. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the sample characteristics.

3.4 It must be stated here that due to the ubiquitous nature of the environmental markers like Galaxolide, Tonalide and various flame retardants combined with very low detection limits of the Agilent 7000 GC/MC QQQ system some level of background contamination may be difficult to avoid. Constant efforts have to be made by all the technicians and analysts involved to keep the background contamination levels as low as possible.

4. DEFINITIONS

4.1. ACCURACY: The closeness of agreement between an observed value and an accepted reference value. When applied to a set of observed values, accuracy will be a combination of a random component and of a common systematic error (or bias) component.

4.2. BATCH: A group of samples, which behave similarly with respect to the sampling or the testing procedures being employed, that are processed as a unit. For QC purposes, if the number of samples in a group is greater than 20, then each group of 20 samples or less will all be handled as a separate batch.

4.3. BIAS: The deviation due to matrix effects of the measured value (Xs - Xu) from a known spiked amount. Bias can be assessed by comparing a measured value to an accepted reference value in a sample of known concentration or by determining the recovery of a known amount of contaminant spiked into a sample (matrix spike). Thus, the bias (B) due to matrix effects based on a matrix spike is calculated as:

B = (Xs - Xu) - K
where:
Xs = measured value for spiked sample,
Xu = measured value for unspiked sample, and
K = known value of the spike in the sample.

Using the following equation yields the percent recovery (%R).

$$\%R = 100\ (Xs - Xu)/\ K$$

4.4. PRACTICAL QUANTITATION LIMIT (PQL): The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. The PQL is generally 3 to 5 times the MDL. However, it may be nominally chosen within these guidelines to simplify data reporting. For many analytes the PQL concentration is selected as the lowest non-zero standard in the calibration curve. Sample PQLs are highly matrix-dependent.

4.5. FIELD DUPLICATES: Independent samples which are collected as close as possible to the same point in space and time. They are two separate samples taken from the same source, stored in separate containers, and analyzed independently. These duplicates are useful in documenting the precision of the sampling process.

4.6. INTERNAL STANDARD: A pure compound added to a sample extract in known amounts and used to calibrate concentration measurements of other compounds that are

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sample components. The internal standard must be a compound that is not a sample component.

4.7. LABORATORY FORTIFIED BLANK/LABORATORY CONTROL SAMPLE (LFB/LCS): A known matrix spiked with compound(s) representative of the target analytes. The LFB is analyzed exactly as a sample. This is used to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

4.8. MATRIX: The component or substrate (e.g., surface water, drinking water) which contains the analyte of interest.

4.9. MATRIX SPIKE: An aliquot of sample spiked with a known concentration of target analyte(s). The spiking occurs prior to sample preparation and analysis. A matrix spike is used to document the bias of a method in a given sample matrix.

4.10. MATRIX SPIKE DUPLICATES: A second aliquot of the same sample used for matrix spike spiked with identical concentrations of target analyte(s). The spiking occurs prior to sample preparation and analysis. They are used to document the precision and bias of a method in a given sample matrix.

4.11. METHOD BLANK: An analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing. The method blank must be carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination resulting from the analytical process. For a method blank to be acceptable for use with the accompanying samples, the concentration in the blank of any analyte of concern shall not be higher than the method detection limit.

4.12. METHOD DETECTION LIMIT (MDL): The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix type containing the analyte. For operational purposes, when it is necessary to determine the MDL in the matrix, the MDL should be determined by multiplying the appropriate one-sided 99% t-statistic by the standard deviation obtained from a minimum of seven analyses of a matrix spike containing the analyte of interest at a concentration one to five times the estimated MDL.

4.13. ORGANIC-FREE REAGENT WATER: All references to water in the method refer to water in which interference is not observed at the method detection limit of the compounds of interest. Organic-free reagent water can be generated by passing tap water through a carbon filter bed containing about 1 pound of activated carbon. A water purification system may be used to generate organic-free de-ionized water.

4.14. PRECISION: The agreement among a set of replicate measurements without assumption of knowledge of the true value. Precision is estimated by means of duplicate analyses. These samples should contain concentrations of analyte above the MDL, and involve the use of matrix spikes or laboratory fortified blanks. The estimates of precision are based on the relative percent difference (RPD).

$$RPD = 100 [(X1-X2) / (X1+X2) /2]$$

where:
X1 and X2 are the two measurements

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4.15. INITIAL CALIBRATION VERIFICATION (ICV): A standard solution from a source, other than normal calibration standards, that is certified and traceable. These standards are used to check the accuracy of a calibration curve.

4.16. CONTINOUS CALIBRATION VERIFICATION (CCV): A standard solution from a source that is certified and traceable. These standards are used to check the accuracy of a calibration curve on daily basis (before the run and every 12 hrs thereafter.

4.17. SPIKE SOLUTION (SS): A solution of method analytes of known concentrations that is used to fortify an aliquot of laboratory reagent water or sample matrix.

4.18. STANDARD CURVE: A plot of concentrations of known analyte standards versus the instrument response to the analyte. Calibration standards are prepared by successively diluting a standard solution to produce working standards, which cover the working range of the instrument. Standards should be prepared at the frequency specified in section six (6) of this SOP. The calibration standards should be prepared using the same type of solvent as the final sample preparation solvent.

4.19. SURROGATE: An organic compound which is similar to the target analyte(s) in chemical composition and behavior in the analytical process, but which is not normally found in environmental samples. Surrogates are used to monitor the preparation, and are added to each field and QC sample prior to extraction.

5. APPARATUS AND EQUIPMENT

5.1 Apparatus.

Agilent 7000 GC/MS triple quadrupole system or equivalent. Must be temperature programmable with an injector port designed for cold splitless injection. A Multi-Mode Inlet (MMI) may be used if available. The GC, GC Automatic Sampler and MSD operating parameters are illustrated in Tables 2, 3 and 4.The analytical column is an HP-5MS UI or equivalent, fused silica capillary, 20 m x 0.25 mm x 0.25 um or similar column connected at midpoint (5/15 split) to an Ultimate Union (for backflushing the primary column after each injection).

5.2 Data system

5.2.1 A computer system is interfaced to the GC/MS QQQ that allows the continuous acquisition and storage on machine-readable media of all chromatographic measurements obtained throughout the duration of the chromatographic run sequence program.

5.2.2 This system currently uses the Masshunter software from Agilent Technologies Inc. For reporting data, the system currently uses DEP LIMS.

5.3 Gases

5.3.1 Helium, UPC Grade, is used as the carrier and collision cell gas. The helium is passed through an oxygen and a hydrocarbon filters before it enters the GC.

5.3.2 Nitrogen, UPC Grade, is used as a collision cell gas. The N_2 is passed through a hydrocarbon filter before entering the GC.

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6. REAGENTS AND CHEMICALS

6.1. Solvents

6.1.1. Methylene Chloride, Fisher Scientific, Pesticide, HPLC or OPTIMA grade.

6.1.2. Iso-octane, Fisher Scientific, Pesticide, HPLC or OPTIMA grade.

6.2. Standards

6.2.1. For detailed standard preparation see APPENDIX

6.3. Stock and Intermediate Standards

6.3.1. Custom made stock standards and/or neat standards for individual analytes (two different sources or two different lot numbers if only one vendor is available) are purchased from commercial suppliers. The concentrations of currently purchased custom made standards are listed in the appendix: "Preparation of environmental tracers standards".

6.3.2. Intermediate standard solutions are mixtures of individual standards used to prepare working standards. They are prepared in our lab from stock standards by mixing / diluting with methylene chloride or acetone. They also can be purchased from a certified manufacturer (ISO 9000 or EPA certified). The concentrations of the individual components in the intermediate standard are typically 10 mg/L. Intermediate standards are replaced once per year. New standard is checked vs. a second source (QC Standard), after diluting it to one of the working standard levels. The criterion of acceptance is +/- 10% difference. All standard preparations and verifications have to be recorded in the Standard Preparation Tracker located in DEP LIMS. A list containing all standards with their serial numbers used in the analysis must be part of analysis documentation folder.

6.4. Working Standard Solutions (Calibration standards)

6.4.1. Working standard solutions are a series of diluted individual or mixture standards in methylene chloride. They are used for calibration and are prepared by diluting intermediate standard solutions with methylene chloride. The internal standard mixture listed below is added to every working standard at100 ug/L.

Internal Standard Mixture:

Benzophenone-d10
Tributyl Phosphate-d27

Triclosan Methyl-d3
Triphenyl Phosphate-d15
Phenanthrene-d-10

6.4.2. The concentrations for each analyte in the working (calibration) standards are 1, 5, 10, 25, 50, 100, 250, and 500 ug/L. The second source/CICV standard is prepared at 50 ug/L.

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6.5. Spiking Solution

6.5.1. These solutions are used when fortifying sample matrix spikes or lab fortified blanks at extraction. These fortified samples are used to verify the accuracy and precision of the sample preparation and analysis.

6.5.2. Spike solutions are prepared by diluting stock or intermediate standards with acetone. Concentrations of the parameters in these solutions are 250 ug/L for all analytes and surrogates.

6.5.3. Freshly prepared spike solutions are checked vs. valid working standards. The acceptance criterion is +/- 20 % difference.

6.6. Initial Calibration Verification Verification (ICV)

6.6.1 Initial Calibration Verification (ICV) standards are prepared from a source, other than calibration standards, that is certified and traceable. These standards are used to check the integrity of the calibration standards and are run and analyzed whenever an initial calibration curve is built. The acceptance criterion is +/- 30% difference.

6.6.2 The Analyst must insure that the calibration standards are checked with a CCV standard before every analytical run and then in 12 hrs intervals thereafter. The acceptance criterion is +-20%

6.7 Analyte protectants.

The use of analyte protectants was proposed by Anastassiades et al. (ref 15-15 and 15-16) and it has become an established practice for pesticides analysis in food products analysis, where triple quad GC/MS/MS technology has found its widest use. Agilent 7000C GC/MS/MS instrument is very sensitive to matrix induced response enhancements which makes exact quantitation problematic.

Analyte protectants are generally very polar organic compounds injected at very high concentrations along with every standard and sample in the analytical run sequence. Due to their polarity, they are thought to deactivate all the active sites in the system, and, due to their very high concentration, to render all the other matrices in the samples basically insignificant. In effect all the samples and standards injected into the GC/MS/MS system have effectively the same matrix – the analyte protectants – and therefore matrix induced response differences are greatly reduced.

This method uses a mixture of three specific analyte protectant compounds: Ethoxyglycerin, Gulonolactone and Sorbitol at 2500 ppm, 2500 ppm and 5000 ppm respectively. Due to solubility considerations the working solution is prepared in MeOH containing 5% of DI water. It is injected along with every standard and sample using a “sandwich injection” technigue. 0.4 uL of the above analyte protectants mixture is injected, which corresponds to 1 ug, 1 ug and 2 ug of the corresponding analyte protectants on column.

7. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

7.1. Samples must be collected in glass bottles with Teflon-lined screw caps.

7.2. All samples must be refrigerated at -4 degrees C from time of collection until extraction.

7.3. All samples must be extracted within 14 days of collection and completely analyzed within 40 days of extraction.

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8. SAMPLE PREPARATION PROCEDURE

8.1. See Preparation SOP GC-002-2: "EXTRACTION OF PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBs) FROM WATER, WASTES, SOIL/SEDIMENT, PUFS AND FILTERS FOR SUBSEQUENT ANALYSIS BY GC OR GC/MS TECHNIQUE" (ref 15.2). 1L water sample are extracted with Methylene Chloride (3x60 mL) and solvent exchanged to isooctane to a final volume of 0.25 mL. 0.2 mL is submitted for analysis to account for the addition of 50 uL of internal standard.

8.2. Extreme care has to be exercised by all the laboratory staff involved in preparation and analysis to limit background contamination with personal care products. Three method blanks are to be extracted with each sample batch to account for variability in background contamination. So called "dry blanks" should be performed unless clean matrix water can be found that consistently shows the same background contamination as the "dry blanks".

8.3. Each sample batch collected in the field should contain at least one field blank, one trip blank and one equipment blank. Matrix water for all three blanks will be provided by the DEP Central Laboratory.

8.4. Water Samples Collection Instruction for the Trace Level Analysis of Tonalide and Galaxolide

8.4.1 Two of the target analytes, galaxolide and tonalide, are contained in personal care products ranging from cosmetics to detergents. The organophosphate flame retardants are found in plastics, textiles, furniture and building materials among many other products. All the environmental tracers covered by this method have a ubiquitous presence in our daily lives, thus creating unique challenges to their analysis. The major analytical issue is to minimize their background level not only in the laboratory environment but also during the field sampling to achieve the lowest possible detection limits. To monitor the background levels during the field sampling, the procedure requires one trip blank, one or two field blanks, and one or two equipment blanks per sampling trip. Two field blanks and two equipment blanks will be required in the case of two or more sampling locations in a trip. These field quality control blanks are described below. To minimizing background level, the samplers should use or wear personal care products as little as possible, and utilize hand gloves provided by the Central Laboratory during the sampling.

8.4.2 **Field Quality Control Blanks.** The primary purpose of these blanks is to trace sources of artificially introduced contamination in the field.

Trip Blank: A lab reagent water sample that is taken from the laboratory to the sampling site and transported back to the laboratory without having been exposed to sampling procedures. The purpose is to assess contamination introduced during shipping and field handling procedures.

Field Blank: A sample of reagent water poured into the sampling container in the field, preserved and shipped to the laboratory with field samples. Reagent water is provided and labeled in the sampling kits. The purpose is to assess contamination from field conditions during sampling.

Equipment (Rinsate) Blank: A sample of reagent water poured over or through decontaminated field sampling equipment prior to the collection of environmental samples. Again, reagent water is provided and labeled in the sampling kits. The purpose is to assess the adequacy of the decontamination

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process. Assess contamination from the total sampling, sample preparation and measurement process, when decontaminated sampling equipment is used to collect samples.

8.4.3 **Organic Lab Staff. Preparation of Trip Blanks and Reagent Water Bottles.** As soon as lab supervisor receives email from supporting staff that the test of interest is requested, lab staff will prepare and provide supporting group Sampling Instruction Manual, 1 trip blank, 3 to 5 bottles of reagent water for use in the field and 2 to 4 empty bottles labeled field or equipment blank. The reagent water bottles will be labeled "Reagent Water".

9. GENERAL DESCRIPTION OF GC/MS QQQ ANALYSIS

9.1. GC/MS QQQ Maintenance and Tuning

9.1.1 DFTPP tune recommended by EPA 8270D is not applicable for Agilent 7000C GC/MS/MS. Instead, as recommended by 8270D paragraph 11.3.1, this SOP modifies the method to follow manufacturer's tuning instructions. GC/MS/MS tuning includes periodically performed autotune as well as a daily tune check.

9.1.2 Initial checking and maintenance of the instrument consists mainly of the following steps:

9.1.2.1 Check the vacuum operation and periodically run an air/water leak test. Oxygen, nitrogen and water have to be found at below 5% of mass 69 level. Air/water leak test must be run each time the vacuum system is vented. It may take several hours (even overnight) of pumping down to bring the air/water content below 5%!

CAUTION: In order to properly perform the air/water leak test nitrogen gas flow to the collision cell has to be turned manually off at the source! After the test, nitrogen gas has to be turned back on!

9.1.2.2 Perform the triple quadrupole GC/MS system verification (tune check) before each run sequence. Print the tune check report and include with the documentation. The tune check requirements are listed in appendix 2. Periodically perform the EI high sensitivity autotune of the GC/MS/MS triple quadrupole system and save the current settings in the autotune file. The autotune has to be perform, however, every time the tune check fails. The report is printed automatically and it should be placed in the autotune reports log. If the autotune criteria are not met repeat the autotune. If still failing, run the autotune from the default values. If that fails the ion source may have to be cleaned. **It is not recommended to attempt manual tune of the instrument without the assistance of the Agilent Technologies' triple quad trained specialist!**

9.1.2.3 Replace the injector liner (Agilent 2 mm dimpled liner part no: 5190-2296 is recommended for cold splitless injection), cut 1-3 inch of the column after running contaminated samples or encountering unusually heavy matrix. Occasional trimming of the column is still necessary even if backflush technology is used.

9.1.2.4 Run an Instrument Blank and look for instrument contamination.

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9.1.2.5 In case the instrument is contaminated raise the furnace temperature to 260 °C and leave it overnight.

9.1.2.6 To perform maintenance of the MMI inlet replace the liner and septum and clean the inside of the inlet body with cotton swabs soaked with methanol and methylene chloride. **Do not use a harsh brush to clean the inside of the inlet body!** To complete the MMI inlet cleaning perform an inlet bake out procedure. Set the inlet mode to split (split ratio 200:1) and let the gas flow for a couple of minutes. Then raise the inlet temperature to 330 °C and the oven temperature to 300 °C. Bake for 1-2 hrs. It is beneficial and recommended to bake the ion source at 350 °C at the same time.

9.1.2.7 The autosampler syringe has to be frequently and thoroughly cleaned to avoid injection repeatability problems. A dirty plunger will cause reproducibility problems, bad injections occurring randomly, and poor peak shapes and calibration problems. Replacing the syringe on regular, frequent basis is highly recommended.

9.1.1.8. Re-check for instrument contamination and if needed repeat the procedure until the instrument is contamination free.

9.2. GC/MS QQQ system Initial Calibration

9.2.1. Analyze the calibration standards at the following levels: 1, 5, 10, 25, 50, 100, 250, and 500 ug/L. At each level, the internal standards should be present at 100 µg/L. The linearity of the calibration curve is defined by an RSD of =/<20% of the Response Factors of the calibration curve, or by a correlation coefficient not smaller than 0.995. If a higher order curve is to be used the correlation coefficient has to be at least 0.995. When new calibration is inspected linear fit is usually tried first, followed by average response factor (%RSD which is equivalent to weighted linear forced through origin), then followed by quadratic fit and finally higher order curves (like power, for which at least seven point calibration is needed). Do not use the "include" origin option in the Masshunter software. "Ignore" origin is preferable to "force" through origin in most cases. Forcing the curve through origin is acceptable whenever ignoring origin produces nonsensical results for small peaks close to MDL (like negative values). When calibration curve fit is selected in the Masshunter software the % calibration drift has to be monitored in order to have all the calibration levels within 70--130% of the expected value. Special care has to be taken to carefully fit the curves at the lower end of the calibration range where the vast majority of problems occur. Weighting of the calibration curve is frequently necessary to accomplish that goal. If the calibration fitting criteria fail, then the instrument system must be corrected. Some possible problems include standard mixture degradation, injection port inlet contamination, analytical column contamination, and active sites in the column or chromatographic system.

9.2.2. Each time the column length is trimmed due to contamination or a new column is installed the retention times for each compound have to be determined from the scan mode. The MRM mode time segments (acquisition windows) have to be then adjusted accordingly. A mid-level standard should be then run to confirm that all the peaks are fully within the acquisition windows. Both the analysis end of the method (Masshunter Quant) as well as the data-gathering end of the method (Masshunter acquisition) must be adjusted. Approximate retention times, the primary quantitation transitions and confirmatory, secondary transitions (if available) are given along with MRM acquisition time segments

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start/stop times in Table 4. The primary and secondary ions should all be present and the relative intensities should agree within ±30% (absolute) of the reference mass spectrum after background subtraction is performed.

9.3 Daily GC/MS Performance and Calibration Check

The initial calibration curve and instrument performance must be verified on each day of analysis by the measurement of one or more check standards.

9.3.1 Degradation/tailing factor check
A modified GC/MS tuning standard is used to perform this task. The standard contains 5 mg/L each of DDT-p,p'and Pentachlorophenol. Benzidine, which is also recommended by method 8270D, is usually not detectable in the presence of analyte protectants. Since the absence of benzidine peak in the chromatograms has no bearing on the chromatographic behavior of the target analytes and surrogates, only pentachlorophenol is used for the tailing factor determination. The degradation check/tailing factor standard is run at the beginning of the analytical run sequence to verify injection port inertness and GC column performance. A full scan version of the acquisition method is used for this purpose. Degradation of DDT to DDD and DDE should not exceed 20%.
The percent breakdown calculation is performed using the following formula (EPA method 8081)

%Dec. DDT = Sum Peak Area (DDE + DDD) / Sum Peak Area (DDE + DDD + DDT) × 100%

The following are the Relative Retention Times for DDD-p,p' and DDE-p,p' (relative to DDT-p,p'):
DDE-p,p' RRT = 0.885
DDD-p,p' RRT = 0.949

Usually it is necessary to perform spectral deconvolution of the chromatograms to remove the analyte protectants "hump" and obtain correct integration values and clean spectra for target analytes.

The tailing factor for pentachlorophenol should not be higher than 2 according to the following equation:

Tailing Factor = BC / AB

Where the peak is defined as follows: AC is the width at 10% height; DE is the height of peak and B is the height at 10% of DE. This equation compares the width of the back half of the peak to the width of the front half of the peak at 10% of the height. If DDT-p,p' degradation and or tailing factors are excessive the injection port maintenance has to be performed until proper chromatographic performance is restored.

9.3.2 Analyze midpoint level standards as continuing calibration check (CCV) standards. These calibration check standards must be analyzed at the start of each run and at 12-hour intervals thereafter. The acceptance criteria for all components are 80-120% recovery. If the CCV fails corrective action must be taken prior to the analysis of samples. When compounds fail, they may still be reported as non-detects (with a "J" qualifier) if it can be demonstrated that there was adequate sensitivity to detect the compounds at the applicable quantitation

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limit. For situations when the failed compound is present, the concentrations in the affected samples must be reported as estimated.

9.3.3 Calibration verification standard must also meet the following criteria:

9.3.3.1 Minimum response factors: The minimum RF of the most common target analytes must be within 50% of the original calibration.

9.3.3.2 Internal standard responses: the peak area for each of the internal standards may not change beyond the factor of two (-50% to +100%) from that of the average internal standard response of all the levels in the most recent initial calibration.

9.3.3.3 Internal standards retention times- the retention times of the internal standards in the calibration verification standard must not drift by more than 30seconds from that of the mid-point standard level of the most recent initial calibration.

9.3.3.4 If any of the requirements mentioned in 9.3.3 are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Some possible problems are standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, active sites in the column or chromatographic system, and a contaminated MS source.

9.4 GC/MS QQQ Sample Analysis.

9.4.1 Quantitative analysis of all analytes is accomplished by the internal calibration method. All target component positives found have to be quantified using at least a five-point calibration curve. Each sample extract MUST contain 100 µg/L of each internal standard. Any extract that is determined, in initial analysis, to contain high concentrations of any analytes should be diluted as deemed necessary. **The concentration of internal standard must remain at 100 µg/L when diluting samples by adding additional internal standard to the extract.**

9.4.2 Analysis Run Sequence.

The analyst will set up an analysis run sequence by placing the GC vials containing the sample extracts and standards on the autosampler tray in the following order or similar:

- Isooctane (to clean the system)
- Instrument Blank (containing internal standards at 100 ug/L)
- DDT degradation, pentachlorophenol performance check
- Environmental tracers calibration set (when initial calibration is run, levels 1-8)
- Environmental tracers ICV initial calibration verification
- Environmental tracers CCV continuing calibration verification
- three extraction blanks
- laboratory fortified blanks (LFB)
- sample matrix spike,
- sample matrix spike duplicate
- samples (for up to 12 hrs)
- Environmental tracers CCV continuing calibration check

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- DDT degradation, pentachlorophenol performance check
- samples (for up to 12 hours)

9.4.3 Print a hard copy of the run sequence and place it in the final data folder.

9.5 Sample Analysis using the Masshunter software.

9.5.1 Examine the quantitation reports soon after the samples have been analyzed. Check all of the calibration check standards analyzed during the run sequence and insure that they met the minimum criteria given above.

9.5.2 The concentration reported for an identified hit in the sample extract, expressed in ug/L, is quantitated using the initial calibration curve. However, the analyst must ensure that all identification criteria are fulfilled and the peak area integration was correctly done. If not, the integration has to be re-done by choosing a new set of integration parameters or by manual integration (see SOP CM-018.1 for lab manual integration).

9.5.3 If the response for any of the compounds exceeds the calibration curve, the extract must be diluted so that the response will be within the limits of the curve, and then reanalyzed. Make sure when performing dilutions to use methylene chloride spiked with the appropriate levels of internal standard so that their response will be the same in the dilution as it was in the original extract.

9.5.4 The internal standard responses and retention times in the calibration check standard must be evaluated immediately after data acquisition. If the retention time for any internal standard changes by more than 0.05 minutes from the last check calibration, then the chromatographic system must be evaluated for either a) chromatographic matrix induced shift or inspected for malfunctions (such as leaks) and corrections must be made. The m/z abundances for all of the internal standards must be within a factor of two (50% to 200%) from the abundance in the daily calibration standard check. Corrective action is indicated if the m/z abundance is outside of these limits. If the holding time for a sample has expired and the internal standard areas are out of the acceptance range, then the final report must be commented and any positives detected must be qualified with a "J" to indicate that the result is an estimate.

9.5.5 Using the Qual software examine the degradation/performance check sample. Calculate DDT decomposition and the tailing factor for pentachlorophenol

9.5.6 Analysis of surrogates.

9.5.5.1 The analyst should monitor the performance of the extraction, cleanup, analytical system, and effectiveness of the method by spiking each sample, QC samples and Method Blanks with the surrogate mix containing Dibutyl butylphosphonate and Terphenyl-d14. The surrogate compounds are expected at 50 µg/L in the sample extracts, Blanks, LFBs, and Matrix Spikes.

9.5.5.2 To determine the acceptance accuracy limits for a surrogate the following procedure should be used (See 15.11): (1) for each sample analyzed, calculate the percent recovery of each surrogate in the sample, (2) once a minimum of thirty samples of the same matrix has been analyzed, calculate the average percent recovery (p), and standard

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deviation of the percent recoveries (s), for each of the surrogates, (3) for a given matrix, calculate the upper and lower control limit for method performance for each surrogate standard as follows:

Upper Control Limit (UCL) = p + ts
Lower Control Limit (LCL) = p – ts
Where "t" stands for Student's coefficient for n-1 degrees of freedom (n = number of measurements).

The control limits are supposed to be checked once per year if enough measurements have been conducted. The acceptance limits of 50-150 % (30-1505 for dibutyl butylphosphonate) will be used until enough statistical data is acquired to perform the necessary calculations.

9.5.5.3 If a surrogate recovery fails, the sample has to be re-extracted and re-analyzed. If the sample exceeded the holding time for extraction, and the recovery is biased high, the re-extraction is not required and the reported hits have to be qualified as estimated (J), with the following comment entered in the Sample Report: "Estimated values for "parameter" are due to surrogate recovery out of control limits". If the recovery is biased low the sample must be re-extracted and re-analyzed.

9.5.6 Analysis of matrix spikes and LFBs

Check the Matrix Spike and Lab Fortified Blank recoveries. They must be within the current acceptance ranges. A comment must be added to the final report explaining any deviation from the routine limits whenever the spikes or surrogates are out of range.

9.5.7 Blank contamination

In the event a positive identification is found in both the sample(s) and the instrument blank, the instrument must be cleaned and the sample(s) rerun/reanalyzed. In the event a positive identification is found in both the sample(s) and the extraction blank then the sample set has to be re-extracted and reanalyzed. If samples passed the holding time, the results will be reported with a "V" qualifier up to ten times the amount found in the blank.

9.5.8 Continuing Calibration

9.5.8.1 After initial calibration, the GC/MS QQQ response is checked on a continuing basis (every 12 hours) by using a continuing calibration check (CCV), middle range Environmental Tracers standard. Criterion of acceptance for continuing calibration check is +/-20 % of initial calibration. If a CCV fails, all the samples followed by this standard, have to be re-run for all positive hits found at concentrations higher than PQL level. If the samples have exceeded the analysis holding time then all hits must be flagged as estimated.

9.5.8.2 If CCVs do not meet method requirements but the associated analytical results are below the detection limit or below the quantifiable threshold (PQL) and no systematic failure is observed (i.e., the failure is deduced to be due to a statistical outlier in a multi-component analysis where results from other CCV components and LFB results do not indicate a systematic bias in the analysis or detector response), an appropriate test

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comment and result qualifier (J) shall be provided to indicate the CCVs are outside control limits. However, when a systematic or system failure is detected or the affected analytical data are above the quantifiable threshold, the analysis shall be re-run if possible. If re-analysis is not an option, the analytical results shall be qualified as estimates (see 15.7 for more details).

9.5.9 Quantitation of positives.
Positives are quantified based on their peak area. The concentration reported for an identified hit in the sample extract, expressed in ug/L, is based on calibration curve of the initial calibration. The Masshunter software used to process data will do the interpolation for the sample extract. However, the analyst must ensure that all identification criteria are fulfilled and the peak area integration was correctly done. If not, the integration has to be corrected by choosing a new set of integration parameters or by manual integration (for manual integration procedure see 15.12). Only extract concentrations are calculated by the processing software. The final analyte concentrations in sample are calculated in the QC Manager during the data upload process software. This is done as follows:

- C_{sample} (ng/L) = (Cx) × ($V_{extract}$) × (DF)/(V_{sample})

  where:

- C_X -The concentration, in ug/L, of the analyte in the sample extract determined by the processing software.

- $V_{extract}$ -The volume of the extract, in mLs (usually 0.25 mLs) recorded in the preparation report.

- DF -The dilution of the sample extract recorded in the dilution report.

- V_{sample} -This is the volume of the water sample taken in extraction, expressed in mLs (usually 1000mL).

10. REPORTING AND DOCUMENTATION

10.1 When analysis is complete, generate a .txt file in the Masshunter software for all the reported samples, QC samples and CCVs. The QC Manager is then used to download the final sample results into the Laboratory Information Management System (LIMS). If a sample has been diluted then make sure that the information was entered properly into the QC Manager. The following convention should be used in QC calculator:

TR_CCV for CCV

SURTR_CCV for surrogate in CCV

SURTR_1234567 for surrogate in sample

The QC manager will convert the data from ug/L to ng/L (LIMS reporting units) and also it will add MDLs for all analytes not included in the text file (nondetects).

10.2 Add the appropriate qualifiers and comments to the sample data in the QC Manager after performing a complete review of the sample data and QC data. Follow DEP SOP OG-003, GC Group Data Review/Authorization Checklist for details of data review and reporting, and Section 11 (QUALITY CONTROL). See DEP SOP LB-027, Standard

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Operating Procedure for Reporting Qualified Data for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option. Definitions of the available qualifying codes are listed in DEP SOP LB-027.

10.3 All quantitation reports for samples and QC should be placed in a results folder labeled with the job number, the sample numbers, and the type of analysis.

10.4 Enter the results for each sample in the analysis results form. Print out QC results from the QC Manager. Include a copy of dilutions page if any. Also include a print out of sample positives from the data review and file it in the data folder.

10.5 Submit the result folder to the GC section supervisor. The supervisor will review results and then authorize the sample. The supervisor will then file the folders in the GC pesticides lab.

11. QUALITY CONTROL

11.1 See DEP SOP GC-001, Quality Assurance/Quality Control in the GC pesticides Laboratory for detailed information on quality control, definitions and interferences. See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option.

11.2 All data generated by a chemistry analyst are subjected to supervisory review and authorization. The supervisor is assessing the validity of data generated by assuring that all QA/QC criteria are met by checking the following review/authorization checklist:

- Check sample preparation report
  - procedure
  - surrogate and spiking solutions
  - preparation holding time
- Check project log-in folder for special requests
- Check initial calibration for linearity and fit
- Check continuing calibration check standards
- Check surrogate recoveries
- Check accuracy and precision for LFB and Matrix Spikes
- Check internal standard recoveries
- Check retention time drift
- Check for blanks contamination
- Check for positives:
  - Proper integration of the peaks (baseline)
  - Retention time confirmation
  - Amount confirmation
  - If response is within the calibration range
  - Calculations
  - Qualifiers
- Check information in the MassHunter Report Header:
  - SOP #
  - Instrument ID
  - Analyst name
- Evaluate the data against any field information

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- Check field preservation record
- Check LIMS report:
  - Preparation and analysis dates
  - MDLs
  - Amounts reported for positives
  - Qualifiers
  - Comments

12. DATA ARCHIVAL

12.1 All raw data are retained in electronic or hard copy format. All paper formats are maintained either in the laboratory office or in a State warehouse for at least ten years. Electronic chromatographic raw data are archived by analysis file ID and instrument ID on dual CDs by the Laboratory Support Section.

12.2 Move the valid data files (tune, ICALS, CCVs, QC and sample data files) soon after collection from the hard disk of the instrument computers to the network drive \\TLHLAB3\GC\BACKUP_GC_TQ-1 (or TQ-2)_. Raw data files on the hard disk of each instrument may be deleted after 3 to 4 months or they are no longer in need.

12.3 Copy the processed data files Quantitation reports (Masshunter .txt files) into \\TLHLAB3\GC\UPLOAD_GC-TQ-1 (or TQ-2) to convert in QC Manager for reporting in LIMS. These files may be deleted after all results are authorized.

13. DETERMINATION OF METHOD DETECTION LIMITS

13.1 The MDLs were established using procedures given in EPA Chapter 40 Part 136, Appendix B of the Federal Register. Also refer to Reference 15.1 and 15.14.

13.2 Seven replicate samples are analyzed near the MDL. The MDL is the concentration of analyte at which there is analyte present with 99% confidence.

13.3 The results of the seven replicate data sets are tabulated and the statistical calculations are performed electronically to establish or update the MDLs and PQLs for each analyte and matrix.

14. SAFETY/HAZARDOUS WASTE MANAGEMENT

See SOP OG-001, Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories) for specific details on safety and hazardous waste management.

15. REFERENCES

15.1 Test Methods for Evaluating Solid Wastes, Third Edition, SW-846, Method 8270D, Semi-volatile Organic Compounds By Gas Chromatography/Mass Spectrometry (GC/MS).

15.2 DEP SOP GC-002-2, : “EXTRACTION OF PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBs) FROM WATER, WASTES, SOIL/SEDIMENT, PUFS AND FILTERS FOR SUBSEQUENT ANALYSIS BY GC OR GC/MS TECHNIQUE” “

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15.3 Florida Department of Environmental Protection, Standard Operating Procedure LB-007 *Procedure and Policy for Method and Instrument Validation.*

15.4 Florida Department of Environmental Protection, Standard Operating Procedure LB-031 *Limit of Detection Verification.*

15.5 Comprehensive Quality Assurance Plan, Chemistry Section, Bureau of Labs, DEP, 2010.

15.6 DEP SOP SV-009. BNA Standard Preparation.

15.7 DEP SOP SV-011. BNA Group Data Review/Authorization.

15.8 DEP SOP LB-027. Standard Operating Procedure for Reporting Qualified Data.

15.9 DEP SOP SV-010. Quality Assurance/Quality Control in the Semi-Volatile Organics Laboratory (BNA-GC/MS).

15.10 DEP SOP OG-001. Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories).

15.11 EPA 8000C Determinative Chromatographic Separation; Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW 846; 3rd Edition, Update 4, January 3, 2008.

15.12 DEP SOP CM-018-1 Laboratory Policy Regarding Manual Chromatographic Peak Integration.

15.13 DEP SOP OG-001-3; Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories).

15.14 Code of Federal Regulations, Title 40, Part 136, Vol. 49, No. 209, Method 625: Base/Neutrals and Acids, 10/26/84.

15.15 Anastassiades, M., Mastovska, K., Lehotay, s.J., "Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides" Journal of Chromatography A, 1015 (2003) 163-184.

15.16 Mastovska, K., Lehotay, S.J., Anastassiades, M., "Combination of analyte protectants to overcome matrix effects in routine GC analysis of pesticides residues in food matrices" anal. Chem. **2005**, 77, 8129-8137.

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16. TABLES

TABLE 1: Internal standard and surrogate associations, MDLs, PQLs and RTs for environmental tracers in water samples.

Analyte	**Approximate Retention Time (min)**	**Associated internal standard**	**MDL ug/L**	**MDL ng/L**	**PQL ug/L**	**PQL ng/L**
			extract	sample	extract	sample
Dibutyl butylphosphonate **surrogate**	6.27	Tributyl Phosphate-d27				
TCEP	7.51	Tributyl Phosphate-d27	4.0	1.0	12	3.0
TCPP	7.86	Tributyl Phosphate-d27	40	10	120	30
TDCPP	13.16	Triphenyl Phosphate-d15	8.0	2.0	24	6.0
Triphenyl Phosphate	13.76	Triphenyl Phosphate-d15	8.0	2.0	24	6.0
Terphenyl-d14 **surrogate**	11.66	Phenanthrene-d10				
Triclosan Methyl	11.10	Triclosan Methyl-d3	0.50	0.13	1.5	0.38
Galaxolide	8.39	Benzophenone-d10	30	7.5	90	23
Tonalide	8.50	Benzophenone-d10	6.0	1.5	18	4.5

TABLE 2: GC/MS Operating Conditions: (METHOD TRACERS_MRM-yymmdd)

Parameter	**Setting**
Mass spec:	70 eV. Peak width 0.7s. detector setting-gain. Source temp. 300 C. Collision cell He: 2.25 mL/min, Nitrogen 1.5 mL/min.
Scan Time:	About 3.3 cycles/s. MRM mode dwell times variable, summing up to about 300 ms per time segment. Gain 20 for each transition monitored.
GC Oven Temperature Program:	50 °C for 1.5 min, to 160 °C at 55 C °/min, hold 0 min, to 240 C at 8 C/min hold 0 min, to 280 °C at 50 c/min, to 290 C at 100 c/min.hold at 290 for 4.0 min. This program is the same for full scan (used for RT shifts determination) or MRM.
Injector operating conditions:	Temp: 60 °C for 0.35 min; 900 C °/min to 280 C. hold for 14 min, then 900 c/min to 310 C hold 0 min Mode: pulse splitless; pulse pressure 30 PSI for 1.4 min after pulse pressure: Constant Flow ON, 2.2738 psi at 60 °C (0.8 mL/min); cryo: ON (nitrogen gas); Cryo use temperature 200 °C
Column 1 configuration	HP-5MS UI, 5m-0.25mm-0.25um; in: front MMI inlet, out aux EPC 4
Column 1 flow	0.8 ml/min for 14 min, then at 100 mL/min to -2.5 mL/min for 1.5 min, then at 100 mL/min to -5 mL/min for until end run

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Column 2 configuration	HP-5MS UI, 15m-0.25mm-0.25um; in: t aux EPC 4; out: vacuum
Column 2 flow	0.9 ml/min for 5.534 min, then at 100 mL/min to 2.4 mL/min until end run
MS interface temperature:	300 °C
Injection Type:	Pulsed Splitless (cold). Pulse pressure 30 PSI for 1.4 min; purge to split vent 50 mL/min at 1.5 min. Gas saver 20 mL/min after 5 min.
Injection Volume:	2µL
Backflash:	Concurrent + end run; see columns flows conditions .
Total Run Time:	18.4 min.

TABLE 3: GC AUTOSAMPLER OPERATING PARAMETERS FOR GCMS-QQQ ANALYSIS

Autosampler Parameter	**Setting**
Syringe size	10 uL
Injection volume	2 uL
Solvent A washes	5 pre- 15 post (DI Water)
Solvent A volume	8 uL
Solvent B washes	5 pre- 15 post (Acetone)
Solvent B volume	8 uL
Sample washes	1
Sample wash volume	4 uL
Sample pumps	7
Dwell time pre-inj	0
Dwell time post-inj	0
Solvent wash draw speed	300 uL/min
Solvent wash dispense speed	6000 uL/min
Sample wash draw speed	50 uL/min
Sample wash dispense speed	6000 uL/min
Injection dispense speed	68uL/min
Viscosity delay	7 sec
Sample depth	disabled
Injection type	3-layer Sandwich
L1 airgap	0.0 uL
L2 Volume	0.2 uL MeOH
L2 airgap	0.0 uL
L3 Volume	0.4 uL analyte protectants
L3 airgap	0.0 uL
Sample overlap	Not enabled

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TABLE 4. MRM method acquisition parameters

Analyte	**Time segment/time segment start time**	**Quantifier transition ions**	**Collision energy (V) quant**	**Qualifier transition ions**	**Collision energy (V) qual**
Benzophenone-d10	TS1/5.80	192-110	9	192-82	32
Dibutyl butylphosphonate	TS1/5.80	138.9-121	10	138.9-82.9	10
Tributyl Phosphate –d27	TS1/5.80	103.1-83.0	20	103.1-63.0	37
TCEP	TS2/7.42	248.9-125	9	248.9-63	13
Phenanthrene-d10	TS2/7.42	188-160.2	28		
TCPP	TS2/7.42	125-99	11	125-80.9	27
Galaxolide	TS3/8.15	243-213	12	243-143	24
Tonalide	TS3/8.15	243-159	15	243-145	20
Triclosan Methyl-d3	TS4/10.92	308.8-254	20	308.8-237	15
Triclosan Methyl	TS4/10.92	301.8-251.9	20	301.8-189	42
Terphenyl-d14	TS4/10.92	244.2-240.2	30	244.2-226.3	30
Triphenyl Phosphate-d15	TS5/13.00	341.1-243.1	11	341.1-223.1	24
Triphenyl Phosphate	TS5/13.00	326-233	11	326-170.1	15
TDCPP	TS5/13.00	190.9-75	10	190.9-154.9	5

TABLE 5. : Analyte concentrations in calibration standards and /CCV/ICV for environmental tracers (ug/L) (Test ID W-TR-TQ). The expected spike concentrations in LFBs/MSs are equal to L5

ANALYTE	INT	L8	L7	L6	L5	L4	L3	L2	L1
Galaxolide	10000	500	250	100	50	25	10	5	1
Tonalide	10000	500	250	100	50	25	10	5	1
Triclosan methyl	10000	500	250	100	50	25	10	5	1
Tcep	10000	500	250	100	50	25	10	5	1
Tdcpp	10000	500	250	100	50	25	10	5	1
Tcpp	10000	500	250	100	50	25	10	5	1
Triphenyl phosphate	10000	500	250	100	50	25	10	5	1
Dibutyl butylphosphonate	10000	500	250	100	50	25	10	5	1
Terphenyl-d14	10000	500	250	100	50	25	10	5	1

Expected ICV/CCV/LFB/Ms concentrations are equal to L5.

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TABLE 6: QC MEASUREMENTS AND ACCEPTACE CRITERIA

#	QC MEASUREMENT	ACCEPTANCE CRITERION	CORRECTIVE ACTION
1	LFB, Matrix Spike precision	≤ 30%	Reanalyze/Re-extract/Flag
2	LFB, Matrix Spike - recoveries	60-140%	Reanalyze/Re-extract/Flag
3	ICV	± 30%	Re-run and recalibrate/Flag
3	CCV	± 20%	Re-run and recalibrate/Flag
4	Surrogate recovery	75-119% for Terphenyl-d14 62-116% for Dibutyl butylphosphonate	Reanalyze/Re-extract/Flag
5	Linearity criterion	20% RSD; 0.995 CC	Use appropriate cal fit / Re-run/ Recalibrate
6	Internal std recovery	50-200%	Reanalyze/recalibrate/flag
7	% cal drift	± 30% of expected value	Change fit/weighing/recalibrate/flag

17. APPENDICES

Standard Preparation Procedure

Stock solutions usually come in 1mL glass vials. Take the vials out of the freezer at least 30min before preparation. Open vials and transfer the entire solution into 1mL GC vials (using Pasteur pipettes). Record the transfer date and keep the vial label.
The Intermediate Standard is prepared by diluting the Stock solution. The Working Level Standards are prepared by diluting the Intermediate Standard.

Surrogate:

Purchased standards:

Components	Concentration mg/L	Source	Comments
2-Fluorobiphenyl, Nitrobenzene-d5, p-Terphenyl-d14	1000	Ultra scientific	Purchased Surrogate mix
Dibutyl Butylphosphonate	Neat	Pfaltz & Bauer	

1000 ppm individual component stock solutions:
Solvent: Methanol

Component	Mass of neat standard added g	V_f mL	Final Conc. mg/L
Dibutyl Butylphosphonate	0.05	50	1000

100 ppm Surrogate Mix Intermediate solution (all components combined):
Solvent: Acetone

Component	Amount of 1000 ppm Stock added uL	V_f mL	Final Conc. mg/L
Purchased Surrogate mix	200	2	100
Dibutyl Butylphosphonate	200		100

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Surrogate Mix spike solution:
Solvent: Acetone

Component	Amount of 100 ppm Stock added uL	Vf mL	Final conc. ug/L
Surrogate mix	25	10	250

Internal standard:

Purchased standards:

Components	Conc. mg/L	Source	Comments
Tri-n-butyl-d27-phosphate	Neat	CDN Isotopes	
Triphenyl Phosphate-d15	Neat	Sigma-Aldrich	
Benzophenone-d10	Neat	Sigma-Aldrich	
1,4 –Dichlorobenzene-d4, Acenaphthene-d10, Chrysene-d12, Naphthalene-d8, Perylene-d12, Phenanthrene-d10	4000	Ultra scientific	Purchased ISTD mix
Triclosan-methyl-d3	100	Crescent Chemical	

Stock solutions prepared from purchased standards:

Component	Mass of neat compound added g	Volume of solution added mL	Vf mL	Solvent	Final conc. mg/L
Triphenyl Phosphate-d15	0.01	-	10	Methanol	1000
Benzophenone-d10	0.025	-	25	Acetone	1000
Tri-n-butyl-d27-phosphate	0.025	-	25	Methanol	1000
Triclosan-methyl-d3	-	0.5	5	Methylene Chloride	10
Purchased ISTD mix	-	0.25	10	Methylene Chloride	100

10 ppm internal standard intermediate (Contains all ISTD components except for Triclosan-methyl-d3. Triclosan-methyl-d3 is added directly to the working standards due to low concentration of the available purchased standard):
Solvent: Methylene Chloride

Component	Conc. of Stock mg/L	Amount of Stock added mL	Vf mL	Final conc. mg/L	Comment
Purchased ISTD mix	100	1.0	10	10	
Benzophenone-d10	1000	0.1		10	
Tri-n-butyl-d27-phosphate	1000	0.1		10	
Triphenyl Phosphate-d15	1000	0.1		10	

Internal standard working solutions
Solvent: Methylene Chloride

Component	Amount of 10 ppm ISTD intermediate added uL	Amount of 10 ppm Triclosan-methyl-d3 solution added uL	ISTD Vf mL	Final conc. ug/L
ISTD mix	500	500	10	500
ISTD mix	100	100	10	100

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Target Analytes:

Purchased standards:

Components	1st source		2nd source	
	Concentration mg/L	Source	Concentration mg/L	Source
Galaxolide	Neat	Penta Manufacturing	Not available, used 1st source	
Tonalide	100	LGC Standards	100	LGC Standards
Triclosan Methyl	Neat	Fluka	10	Crescent Chemical
TCEP	Neat	TCI America	Neat	Aldrich
TDCPP	1000	Absolute Standards	Neat	Sigma-Aldrich
TCPP	Neat	Pfaltz & Bauer	Neat	Fluka Analytical
TBP	1000	Restek	1000	Crescent Chemical
TPP	Neat	Chem Service	1000	Crescent Chemical

Individual analyte stock solutions:

Component	Source	Mass of neat compound added g	Volume of solution added mL	Vf mL	Solvent	Final conc. mg/L
Galaxolide	1st	0.0332	-	50	Methylene Chloride	664
Triclosan Methyl	1st	0.0115	-	10	Acetone	1150
TCEP	1st	0.05	-	50	Methanol	1000
TDCPP	1st	-	1	10	Acetone	100
TCPP	1st	0.05	-	50	Methanol	1000
TPP	1st	0.05	-	50	Methanol	1000
TCEP	2nd	0.1	-	10	Methanol	10000
TCEP	2nd	-	0.5	50	Acetone	100
TDCPP	2nd	0.05	-	50	Methanol	1000
TDCPP	2nd	-	1.0	10	Acetone	100
TCPP	2nd	0.0376	-	25	Methanol	1500
TCPP	2nd	-	0.5	5	Acetone	150
TBP	2nd	-	0.5	5	Acetone	100
TPP	2nd	-	0.5	5	Acetone	100

10 ppm Target Analyte Mix Intermediate:
Solvent: Methylene Chloride

Component	Conc. of Stock mg/L	Amount of Stock added mL	Vf mL	Final conc mg/L	Comment
Surrogate Mix	100	0.5	5	10	
Caffeine	1000	0.05		10	
Galaxolide	664	0.075		10	
Tonalide	100	0.5		10	Purchased stock
TBP	1000	0.05		10	Purchased stock
Triclosan Methyl	1150	0.0435		10	
TPP	1000	0.05		10	
TDCPP	100	0.5		10	
TCEP	1000	0.05		10	
TCPP	1000	0.05		10	

Calibration Standards:
Solvent: Methylene Chloride

Calibration level	Vf mL	Amount of 10 ppm Target Analyte Mix Intermediate added uL	Amount of 10 ppm INTERNAL STD Intermediate added uL	Amount of 10 ppm Triclosan-Methyl-d3 std. added uL	Target Analyte Conc. ug/L
L1	10	1	100	100	1
L2	10	5	100	100	5

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L3	10	10	100	100	10
L4	10	25	100	100	25
L5	10	50	100	100	50
L6	10	100	100	100	100
L7	10	250	100	100	250
L8	10	500	100	100	500

5 ppm Galaxolide/Tonalide QC Intermediate:
Solvent: Acetone

Component	Conc. of Stock mg/L	Amount of Stock added uL	Vf mL	Final conc. mg/L	Comment
Galaxolide	664	38	5	5	1st source
Tonalide	100	250		5	Purchased stock

1 ppm QC Intermediate Mix (includes all QC components, except for Galaxolide and Tonalide):
Solvent: Acetone

Component	Conc. of Stock mg/L	Amount of Stock added uL	Vf mL	Final conc. mg/L	Comments
TDCPP	100	100		1	
Tributylphosphate	100	100		1	
Triclosan Methyl	10	1000		1	Purchased stock
Triphenylphosphate	100	100		1	
TCEP	100	100		1	
TCPP	150	67		1	

Spike solution:
Solvent: Acetone

Component	Conc. of Stock mg/L	Amount of Stock added mL	Vf mL	Final conc. ug/L
Galaxolide/Tonalide QC Intermediate	5	1	10	500
QC Intermediate Mix	1	2.5		250

50 ppb CCV standard:
Solvent: Methylene Chloride

Component	Conc. of Stock mg/L	Amount of Stock added uL	Vf mL	Final conc. ug/L
Galaxolide/Tonalide QC Intermediate	5	100	10	50
QC Intermediate Mix	1	500		50
Internal Standard Intemediate	10	100		100
Triclosan-methyl-d3	10	100		100
Surrogate Mix Intermediate solution	100	5		50

Solvent: Methylene Chloride

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GC/MS/MS tune check

Triple Quadrupole GC/MS System Verification - Tune

Instrument Name	ERLN 3 / US93940310	**MS Model**	7000
Tune Date & Time	5/13/2013 9:07:50 AM		
Tune File	C:\MassHunter\GCMS\1\7000\atunes.eiex.tune.xml		

Instrument Actuals

Ionization mode	EI+	Rough Vacuum	1.52E+2 mTorr
Source Temperature	300 °C	High Vacuum	6.61E-5 Torr
Quad. 1 Temperature	150 °C	Turbo Speed	100.0 %
Quad. 2 Temperature	150 °C	Turbo Power	41.524 W
Emission Current	35 uA		

MS1 Checktune Results	Value	Limit	Result
Low mass assignment (target 69.00, actual 68.90)	0.10	<= 0.20	OK
Mid mass assignment (target 264.00, actual 263.90)	0.10	<= 0.20	OK
High mass assignment (target 502.00, actual 501.80)	0.20	<= 0.20	OK
Low mass isotope position (target 70.00, actual 70.00)	0.00	<= 0.20	OK
Mid mass isotope position (target 265.00, actual 264.90)	0.10	<= 0.20	OK
High mass isotope position (target 503.00, actual 502.87)	0.13	<= 0.20	OK
Low mass isotope ratio	1.06%	>= 0.5% and <= 1.6%	OK
Mid mass isotope ratio	5.79%	>= 4.2% and <= 6.9%	OK
High mass isotope ratio	9.92%	>= 7.9% and <= 12.3%	OK
Ratio of mid mass to low mass	13.37%	>= 5.0%	OK
Ratio of high mass to low mass	2.53%	>= 0.8%	OK
Low mass precursor ratio	0.10%	<= 3.00%	OK
Mid mass precursor ratio	0.12%	<= 6.00%	OK
High mass precursor ratio	0.08%	<= 12.00%	OK

MS2 Checktune Results			
Low mass assignment (target 69.00, actual 69.10)	0.10	<= 0.20	OK
Mid mass assignment (target 264.00, actual 264.10)	0.10	<= 0.20	OK
High mass assignment (target 502.00, actual 502.05)	0.05	<= 0.20	OK
Low mass isotope position (target 70.00, actual 70.15)	0.15	<= 0.20	OK
Mid mass isotope position (target 265.00, actual 265.10)	0.10	<= 0.20	OK
High mass isotope position (target 503.00, actual 503.05)	0.05	<= 0.20	OK
Low mass isotope ratio	1.07%	>= 0.5% and <= 1.6%	OK
Mid mass isotope ratio	5.75%	>= 4.2% and <= 6.9%	OK
High mass isotope ratio	10.29%	>= 7.9% and <= 12.3%	OK
Low mass precursor ratio	0.01%	<= 3.00%	OK
Mid mass precursor ratio	0.00%	<= 6.00%	OK
High mass precursor ratio	0.03%	<= 12.00%	OK

Detector			
EMV	1273	<= 2900	OK
Maximum gain factor	16819	>= 100	OK

Air and Water Check	Abundance	Relative Abundance	Limit	Result
PFTBA (69.00)	998695			
Water	3247	0.33%	<= 20.00%	OK
Oxygen	4076	0.41%	<= 2.50%	OK
Nitrogen	15189	1.52%	<= 10.00%	OK

* Nitrogen values are calculated from oxygen abundance

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Appendix of Significant Changes

06/30/2014

1) Caffeine, Atrazine and Tertbuthylazine along with corresponding surrogates and internal standards have been removed from the test.
2) GC program have been modified to include the backflash procedure.
3) Addition of analyte protectants/ sandwich injection technique.
4) Addition of DDT degradation/pentachlorophenol tailing factor chapter.
5) Addition of GC/MS/MS tune check
6) Many editorial changes throughout the text.

06/30/2015

1) Agilent GC/MS/MS 7000B system changed to 7000C system
2) preparation refers to GC-002-2
3) updated MDLs (Table 1)
4) updated QC recovery limits (spikes and surrogates) (Table 6)

06/14/2016

1) Updated surrogate recovery limits
2) Limits for calibration drift check set to 70-130% by EPA 8270D
3) “Include zero” option prohibited for calibration curves. Ignoring zero preferred option.

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